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Hemopoiesis in the splenectomized-pregnant mouse following low-dose total-body irradiation

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20. ABSTRACT (continued)

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Hemopoiesis in the Splenectomized-Pregnant Mouse Following Low-Dose Total-Body Irradiation

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The effect of splenectomy (SPLX) and total-body irradiation (TBI) (50–200 rad) on virgin and pregnant mouse hemopoiesis was studied, using peripheral blood hemogram values and femoral marrow hemopoietic progenitor cell activity (i.e., CFU_E, BFU_E, and GM-CFC). The SPLX-maternal red cell counts and hematocrit values were lower than those of SPLX-virgin mice, reflecting the anemia of pregnancy. But the white cell counts of both SPLX-virgin and SPLX-day-14.5 pregnant mice were significantly higher ($P < 0.005$) than normal-virgin mice. Both nonirradiated and day-4 irradiated SPLX-maternal marrow Ep-independent and Ep-dependent CFU_E were higher than the nonirradiated and day-4 irradiated SPLX-virgin values (respectively, for each TBI dose studied). On the other hand, nonirradiated and day-4 irradiated SPLX-maternal GM-CFC were lower than the nonirradiated and day-4 irradiated SPLX-virgin GM-CFC values. The data demonstrate the potential of the SPLX-maternal femoral marrow to respond to the stress of low-dose TBI with effective compensatory erythropoiesis, possibly at the expense of granulopoiesis.

Key words: splenectomy – irradiation – hemopoiesis

The spleen serves as the secondary locus for fetal hemopoiesis during the later phase of the hepatic stage of hematopoietic ontogeny. The bone marrow takes on the role of the primary site of blood cell formation during the myeloid stage, which continues throughout postnatal and adult life of all mammals. Unlike the spleen of humans or large animals, the mouse spleen continues to be hemopoietically active, and it is an important source of compensatory and auxiliary hemopoiesis during any disturbance of the normal physiologic condition (i.e., total-body irradiation, hypoxia, bleeding, chemotherapy, pregnancy, etc.).

Studies from our laboratory (1), as

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well as others (2–7), have shown that the changes in homeostatic and immunologic regulation in the pregnant mouse include splenomegaly, anemia, leukopenia, and a shift in erythropoiesis from the marrow to the spleen. Similarly, the consequences of splenectomy on murine medullary and extramedullary hemopoiesis have been demonstrated, especially regarding the humoral and cellular responses to various stimuli (8–11). During days 11 to 14 of mouse pregnancy, splenic erythropoietic activity reaches peak compensatory activity while marrow erythropoiesis significantly declines (2,3,7). The present studies were designed to evaluate the compensatory potential of murine bone marrow in the day-10.5 to day-14.5 splenectomized-pregnant animal to support hemopoeisis after exposure to cobalt-60 radiation. The peripheral blood hemogram and marrow erythroid- and granulocytic-progenitor cells were monitored and served as indicators of hemopoietic perturbations after total-body, low-dose irradiation (i.e., 50 to 200 rad).

MATERIALS AND METHODS

Experiments were designed to study the ability of bone marrow to support hemopoiesis in splenectomized-virgin and splenectomized-maternal mice following exposure to low-dose gamma radiation. Eleven groups of mice were established using the following protocol: Young adult virgin female mice (6–7 weeks old) were splenectomized (SPLX) at the same time; 6 to 8 weeks later a group of mice were mated; on day 10.5 of pregnancy a group of mice received a bilateral total-body irradiation (TBI) exposure of 0, 50, 100, 150, or 200 rad from the AFRRI cobalt-60 gamma radiation source at a rate of 40 rad/minute. Both SPLX-virgin and SPLX-pregnant mice were sacrificed for study at 4 days after irradiation (i.e., day 14.5 of pregnancy). The peripheral blood hemogram and bone marrow hemopoietic progenitor cell activity of all mice were assessed as follows: normal-virgin, SPLX-virgin, irradiated-SPLX-virgin, SPLX-day-14.5 pregnant, and irradiated-SPLX-day-14.5 pregnant mice.

Animals. C57BL/6J female mice (Jackson Labs, Bar Harbor, Maine), free of lesions of murine pneumonia complex and of oropharyngeal *Pseudomonas* spp., were maintained on a diet of Wayne Lab-Blox food pellets and acidified water (pH 2.5) made available ad libitum. Mice were housed in small cages (5 mice/cage) in a room with a 12-h light-dark cycle. Splenectomy was performed under light anesthesia and aseptic conditions. Blood was obtained for hematocrit (Hct), red blood cell (RBC) count, and white blood cell (WBC) count from the retroorbital sinus plexus of lightly anesthesized mice, before being sacrificed by cervical dislocation. For each of the 11 groups studied, both femurs were removed from three mice, and the flushed-out marrow was pooled in chilled Supplemented Alpha Modification of Eagle's Medium (SAM) (1) with 2% fetal bovine serum (heat-inactivated at 56°C for 30 min).

In vitro assays. The bone marrow cell suspensions were plated in microplasma clot cultures (1) to assess erythroid progenitor cell activity and in double-layer soft agar cultures (12) to study granulocyte-macrophage progenitor cell activity.

One-ml plasma clot cultures consisted of the following ingredients maintained on ice: 0.1 ml cell suspension (50,000 nucleated cells), 0.3 ml fetal bovine serum (heat-inactivated), 0.1 ml beef embryo extract, 0.1 ml 10% bovine serum albumin, 0.1 ml L-asparagine (final concentration 0.02 mg/ml), 0.1 ml 10^{-4} M-2-mercaptoethanol, and 0.1 ml erythropoietin (Ep) (anemia sheep plasma, step III, Connaught Labs, Swiftwater, Pennsylvania, lot no. 3023-3, 6.7 U/mg protein). Immediately before plating, 0.1 ml bovine citrated plasma, maintained at 37°C, was added to the above mixture. For each prepared cell suspension, six 0.1-ml microtiter well cultures were plated and incubated at 37°C with humidified 5% CO_2 in air. Colonies of eight or more benzidine-stained positive cells were counted as erythroid-colony-forming units (CFU_E) from clots with 0.25 U Ep/ml and harvested at 48 h of culture (13). Several clusters of benzidine-stained positive cells were counted as the younger erythroid-burst-forming unit (BFU_E) from clots with 3.0 U Ep/ml and harvested at 9 days of culture.

The double-layer soft agar culture procedure described by MacVittie (12) was used to study the granulocyte-macrophage colony-forming cell

TABLE 1

Peripheral blood hemogram of virgin female, splenectomized (SPLX)-virgin female, and splenectomized-day-14.5 pregnant C57BL/6J mice[a]

	Virgin	SPLX-virgin	SPLX-pregnant
Red blood cells ($\times 10^6$/mm^3)	8.56 ± 0.06	8.39 ± 0.17	7.28 ± 0.20[b]
Hematocrit (%)	47.0 ± 0.0	47.3 ± 0.5	40.8 ± 0.8[b]
White blood cells ($\times 10^3$/mm^3)	4.38 ± 0.68	9.61 ± 0.71[b]	8.74 ± 1.05[b]

[a] Values expressed as mean ± SEM.

[b] Values significantly different ($P < 0.005$) from normal-virgin female mice.

(GM-CFC). Pregnant mouse uteri extract (PMUE) (2.5% v/v) served as the source of colony-stimulating factor. Cultures of 5×10^4 bone marrow cells plated per ml were incubated at 37°C with humidified 5% CO_2 in air. Colonies (>50 cells) were counted after 10 days of incubation and were considered to be derived from GM-CFC.

Statistical analyses. The data reported from four replicate experiments are indicated as the mean values with standard errors for each group. The two-tailed Student's *t* test was used to determine the statistical significance of group values.

RESULTS

Peripheral blood hemogram. SPLX-virgin mice, 8–10 weeks after surgery, RBC and Hct values were about the same as the normal-virgin mice counts (Table 1). However, values of day-14.5-pregnant mice splenectomized at the same time were significantly lower ($P < 0.005$), reflecting the anemia of the pregnant mice (Table 1). The WBC values of both SPLX-virgin and SPLX-pregnant mice were significantly higher ($P < 0.005$) than normal-virgin mice (Table 1). Four days after TBI there were no differences in RBC or Hct counts between the nonirradiated- and irradiated-SPLX-virgin animals, or between nonirradiated- and irradiated-SPLX-pregnant mice. Each of the SPLX-pregnant mice RBC and Hct counts was lower than those of the SPLX-virgin animals (Fig 1 and 2). With each dose of TBI, both SPLX-virgin and

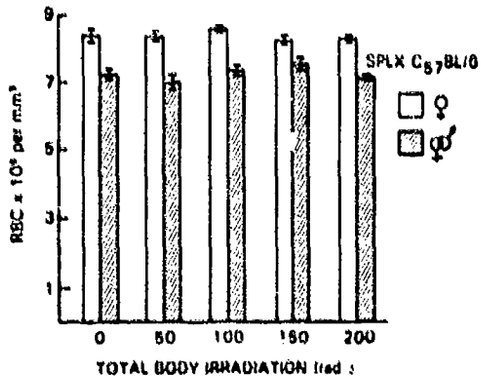

Figure 1. Mean ± SEM values for peripheral blood red cell counts of splenectomized-virgin (♀) and splenectomized-day-14.5-pregnant (♀) C57BL/6J mice, 4 days after total-body irradiation.

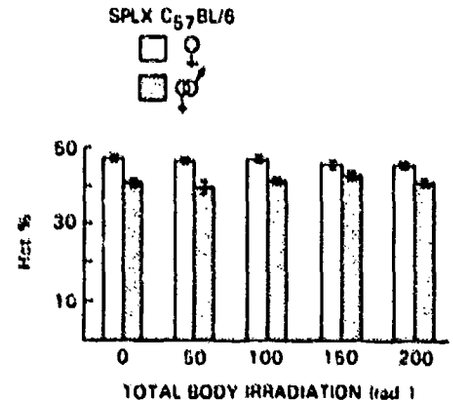

Figure 2. Mean ± SEM values for peripheral blood hematocrit values of splenectomized-virgin (♀) and splenectomized-day-14.5-pregnant (♀) C57BL/6J mice, 4 days after total-body irradiation.

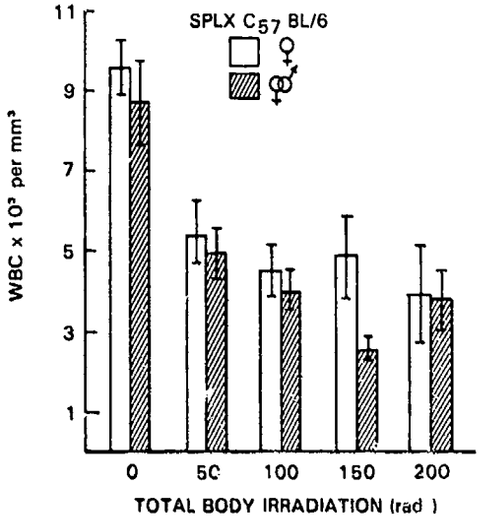

Figure 3. Mean ± SEM values for peripheral blood white cell counts of splenectomized-virgin (♀) and splenectomized-day-14.5-pregnant (♀) C57BL/6J mice, 4 days after total-body irradiation.

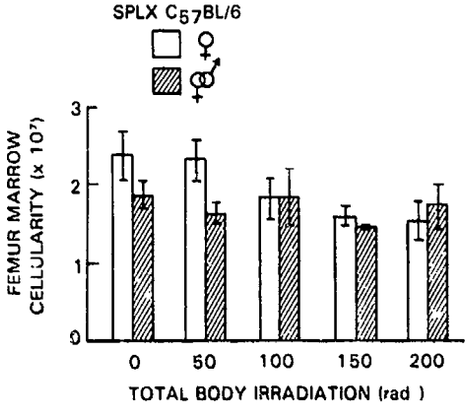

Figure 4. Mean ± SEM values for femur marrow cellularity of splenectomized-virgin (♀) and splenectomized-day-14.5-pregnant (♀) C57BL/6J mice, 4 days after total-body irradiation.

SPLX-pregnant WBC counts 4 days after exposure were significantly lower ($P < 0.005$) than the nonirradiated-SPLX-mice (Fig 3).

Bone marrow cellularity. Splenectomy caused a 55% increase in the virgin femoral marrow cellularity and a 20% increase in the day-14.5 pregnant marrow compared to the normal-virgin marrow (Table 2). The data in Fig 4 reflect slight changes in day-4 bone marrow cellularity of the irradiated-SPLX-mice. Whereas doses of 100 rad and higher reduced the mean SPLX-virgin marrow cellularity values, there were no relative changes noted with SPLX-pregnant mice.

Marrow erythroid progenitor cells. The mean values of two populations of marrow CFU_E in SPLX-virgin and SPLX-pregnant mice are compared with

TABLE 2

Comparison of marrow cellularity and hemopoietic progenitor cells per femur in virgin-female, splenectomized (SPLX)-virgin-female, and splenectomized-day-14.5 pregnant-female C57BL/6J mice[a]

	Virgin	SPLX-virgin	SPLX-pregnant
Femur cellularity ($\times 10^7$)	1.55 ± 0.17	2.4 ± 0.3[b]	1.8 ± 0.1
CFU_E, no Ep ($\times 10^4$)	0.06 ± 0.03	0.1 ± 0.1	0.9 ± 0.5[b]
CFU_E, 0.25 U Ep/ml ($\times 10^4$)	6.8 ± 1.6	9.2 ± 2.7	13.0 ± 3.8[b]
BFU_E, 3.0 U Ep/ml ($\times 10^4$)	1.5 ± 0.3	2.2 ± 0.1[b]	1.5 ± 0.5
GM-CFC ($\times 10^4$)	2.9 ± 0.5	2.8 ± 1.3	2.6 ± 1.1

Erythroid progenitor cells were assayed in microplasma clot cultures with 0.25 U Ep/ml for day-2 CFU_E and 3.0 units Ep/ml for day-9 BFU_E. Pregnant mouse uteri extract was used as colony-stimulating factor in double-layer soft agar cultures for GM-CFC.

[a] Values expressed as mean ± SEM.

[b] Values significantly different ($P < 0.05$) from normal-virgin female mice.

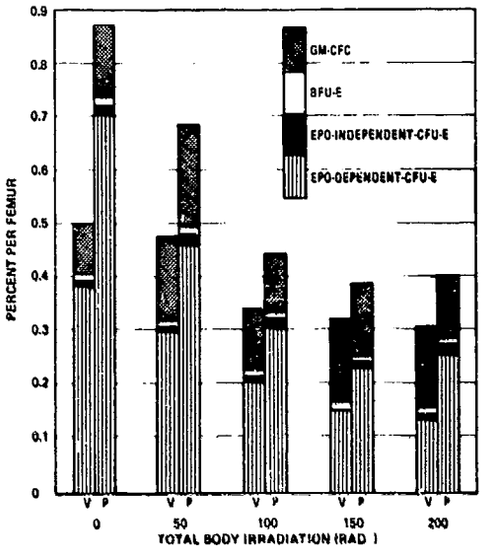

Figure 5. C57BL/6J SPLX-virgin (V) and SPLX-day-14.5-pregnant (P) mean percent values per femur of hemopoietic progenitor cells 4 days after TBI: Ep-dependent-CFU_E, Ep-independent-CFU_E, BFU_E, and GM-CFC.

normal-virgin mice in Table 2. SPLX-pregnant mice Ep-independent and Ep-dependent CFU_E were significantly greater than values of normal mice ($P \leq 0.05$). The mean values of both populations of CFU_E from nonirradiated and irradiated SPLX-pregnant mice were greater than the values from nonirradiated and irradiated SPLX-virgin mice (Fig 5). The effect of TBI on marrow erythropoiesis is demonstrated in Fig 5 by the changes in the percent CFU_E/femur of both SPLX-virgin and SPLX-day-14.5 pregnant mice with each exposure dose. Four days after each TBI dose, there was a decrease in SPLX-maternal and SPLX-virgin femur CFU_E. The mean SPLX-virgin CFU_E/femur value for each group was lower than the mean SPLX-maternal CFU_E/femur value at the same dose. Splenectomy resulted in a 46% increase in virgin mice total marrow BFU_E values. The SPLX-day-14.5 maternal values were similar to those of normal virgin mice but lower than the SPLX-virgin mice values (Table 2). The day-14.5 irradiated SPLX-maternal percent BFU_E/femur values were lower than the irradiated-SPLX-virgin values (Fig 5).

Marrow granulocyte-macrophage progenitor cells. There was no difference between the total marrow GM-CFC values of normal-virgin, SPLX-virgin, and SPLX-maternal mice (Table 2). There was a more appreciable decrease in the % GM-CFC/femur 4 days after each dose in the SPLX-maternal groups compared to the SPLX-virgin groups (Fig 5). The SPLX-maternal mean GM-CFC/femur values were lower than those of the SPLX-virgin group values.

DISCUSSION

Our experiments were designed to evaluate the compensatory potential of the mouse bone marrow. Stress conditions such as splenectomy, pregnancy, and total-body irradiation, which change the physiological steady-state condition, appeared to initiate competition between granulopoiesis and erythropoiesis in a hemopoietic microenvironment known to be conducive to granulopoiesis.

Our data for the virgin mouse are in accordance with earlier reports showing that if the spleen were removed and if the mouse were subjected to different stimuli (i.e., irradiation, hypoxia, etc.), there would be a diminished in vivo responsiveness of marrow to Ep (9), as well as an increase in granulopoietic activity, at the sacrifice of erythropoiesis (8). In our hands, surgical removal of the spleen produced a significant increase in the virgin-mouse peripheral

blood WBC counts and in both marrow erythroid progenitor cells (CFU_E & BFU_E) but no change in marrow GM-CFC. Four days after TBI, the mean % GM-CFC/femur values were higher in the SPLX-virgin groups compared to the SPLX-pregnant mice. As expected from our previous studies (1), there was a much greater reduction in both CFU_E and BFU_E values of SPLX-virgin mice with each dose of radiation exposure, compared with the granulocytic progenitor cell values. Values for the SPLX-pregnant mouse peripheral blood hemogram and bone marrow hemopoietic progenitor cells reflected the anemia and a medullary shift from granulopoiesis to enhanced erythropoiesis, with the appearance of a subpopulation of Ep-independent-CFU_E. These studies suggested that the maternal bone marrow can support erythropoiesis stimulated by pregnancy-induced anemia in the absence of the spleen.

Studies from our laboratory (1) showed that low-dose TBI to the day-10.5 intact pregnant mouse caused a further suppression in recovery of the day-14.5 medullary erythropoiesis, with a concomitant and significant increase in maternal spleen erythropoietic activity. Potential of the SPLX-maternal marrow to respond to the stress of low-dose gamma radiation was demonstrated in the present studies, with effective compensatory erythropoiesis.

Appearance of an Ep-independent population of CFU_E in the SPLX-day-14.5 maternal marrow was not expected, especially since these cells were not observed in our previous studies with irradiated-pregnant mice (1) or in studies done by Rich and Kubanek (7,14). Furthermore, maternal splenic erythropoiesis, which resulted in a 40-fold increase in CFU_E numbers, did not exhibit a change in Ep sensitivity at any time during the stimulated period of pregnancy. During ontogenesis, day-14.5-fetal liver CFU_E are not only more sensitive to Ep than adult bone marrow CFU_E but also consist of a subpopulation of Ep-independent-CFU_E (7,15). Unlike the fetal liver, the SPLX-maternal-marrow Ep-independent-CFU_E were radiosensitive to gamma radiation. Commencing with 50 rad, there was a reduction in the mean percent Ep-independent-CFU_E/femur, and with each higher dose there was a further decrease in each respective value. Although velocity sedimentation studies by Rich and Kubanek (14) clearly documented a homogeneous CFU_E population from adult bone marrow or whole fetal liver, with respect to their Ep sensitivity, we observed two populations of CFU_E in the marrow of nonirradiated- and irradiated-SPLX-pregnant mice.

There is an obvious difference between the SPLX-pregnant marrow and the normal-pregnant marrow. Still unclear is the underlying mechanism that caused the change in marrow microenvironment from a site predominantly granulopoietic in the virgin mouse and pregnant mouse to a site of increased erythropoietic activity in the SPLX-maternal mouse. Perhaps removal of the spleen causes a change in the regulatory mechanism for erythropoiesis (i.e., absence of an inhibitor factor produced by the spleen). Inasmuch as the mouse is unique with a hemopoietically active spleen during adult life, studies are presently being conducted in our laboratory with a dog model to understand further the regulatory mechanisms operative in the normal and the perturbed physiologic steady-state condition.

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